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GB 1374379
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(58) Field of search
C3H

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(54) The tripeptide eisenin and carcinostatic compositions having an immunopotentiating carcinostatic effect which contain it

(57) The tripeptide Eisenin of the general formula

L-Pyroglu-L-Glu-l-Ala

wherein Pyroglu, Glu and Ala represent pyroglutamic acid, glutamic acid and alanine respectively, found in the seaweed *Eisenia bicyclis* and obtainable synthetically is a carcinostatic agent having an immunopotentiating carcinostatic effect.

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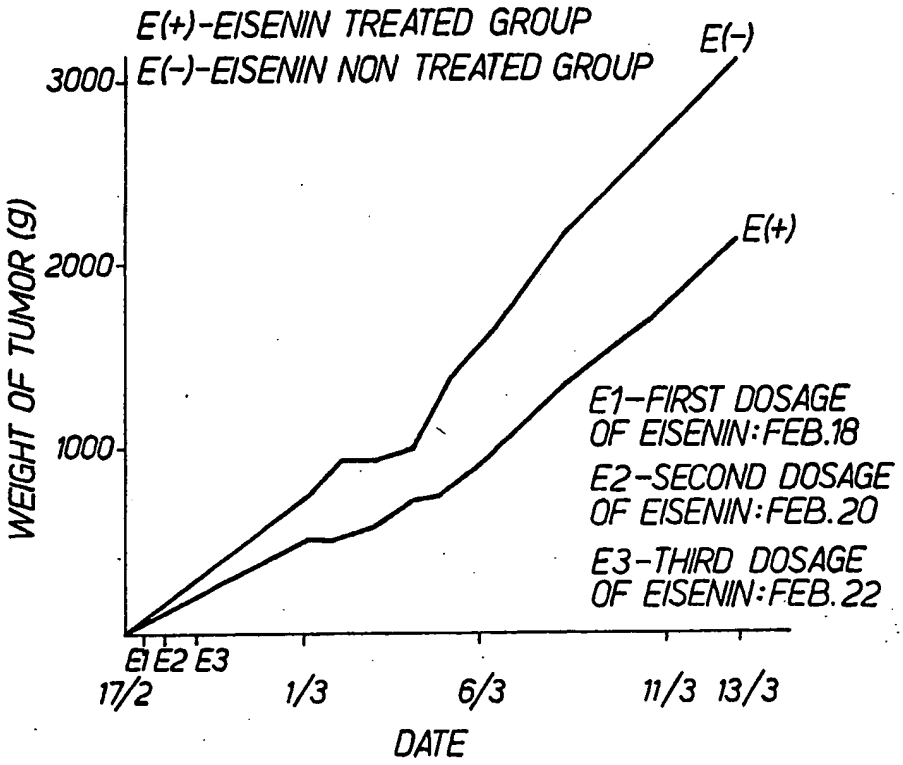
E(+)-EISENIN TREATED GROUP
E(-)-EISENIN NON TREATED GROUP
E(-)
E(+)
3000
2000
1000
WEIGHT OF TUMOR (g)
E1-FIRST DOSAGE OF EISENIN: FEB.18
E2-SECOND DOSAGE OF EISENIN: FEB.20
E3-THIRD DOSAGE OF EISENIN: FEB.22
E1 E2 E3
17/2
1/3
6/3
11/3
13/3
DATE

FIG. 1.

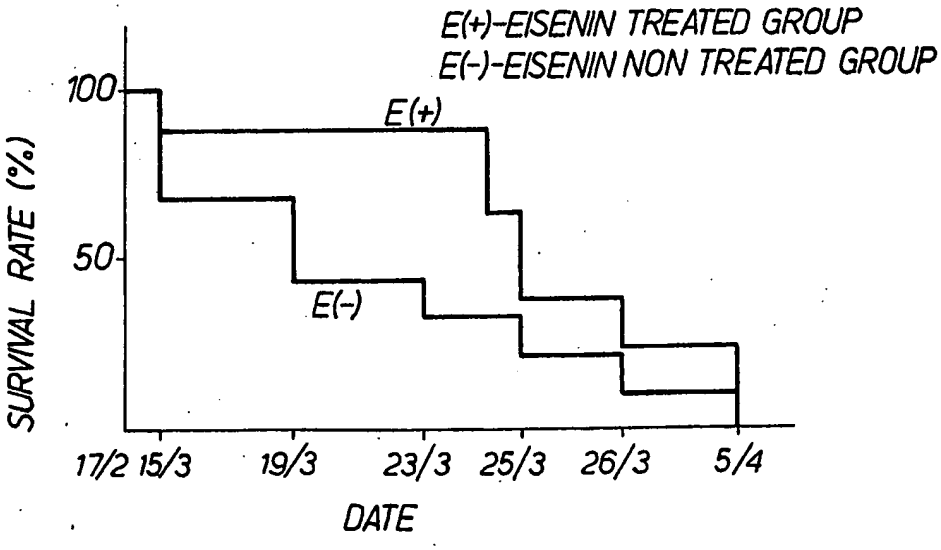
E(+)-EISENIN TREATED GROUP
E(-)-EISENIN NON TREATED GROUP
100
50
SURVIVAL RATE (%)
E(+)
E(-)
17/2
15/3
19/3
23/3
25/3
26/3
5/4
DATE

FIG. 2.

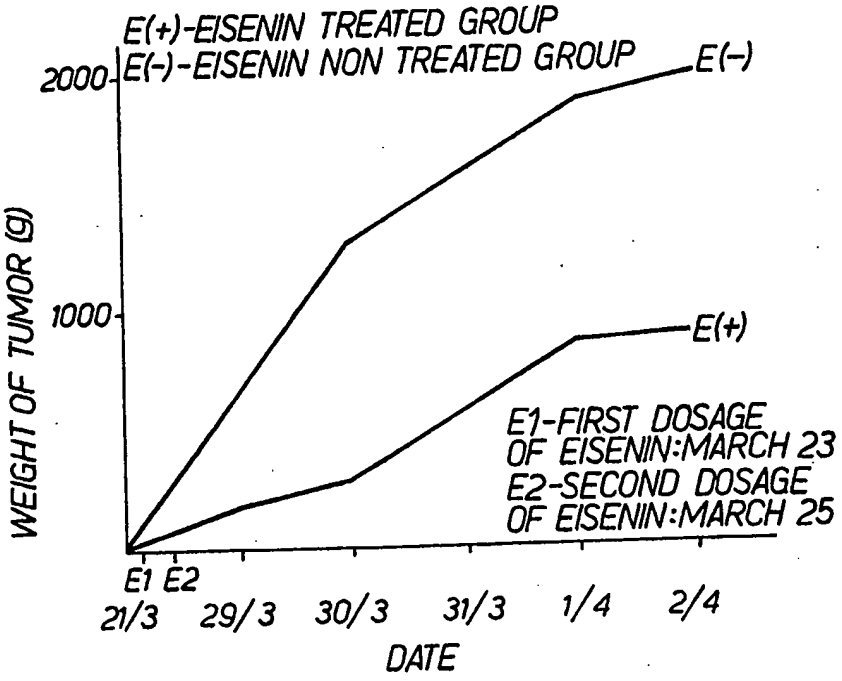
E(+)-EISENIN TREATED GROUP
E(-)-EISENIN NON TREATED GROUP
2000
1000
WEIGHT OF TUMOR (g)
E(-)
E(+)
E1-FIRST DOSAGE OF EISENIN:MARCH 23
E2-SECOND DOSAGE OF EISENIN:MARCH 25
E1 E2
21/3
29/3
30/3
31/3
1/4
2/4
DATE

FIG.3.

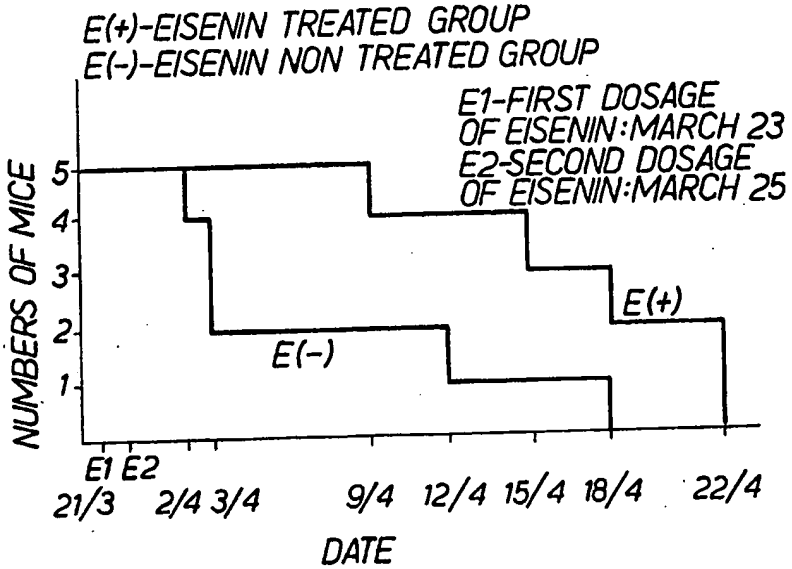
E(+)-EISENIN TREATED GROUP
E(-)-EISENIN NON TREATED GROUP
E1-FIRST DOSAGE OF EISENIN:MARCH 23
E2-SECOND DOSAGE OF EISENIN:MARCH 25
NUMBERS OF MICE
5
4
3
2
1
E(+)
E(-)
E1 E2
21/3
2/4
3/4
9/4
12/4
15/4
18/4
22/4
DATE

FIG.4.

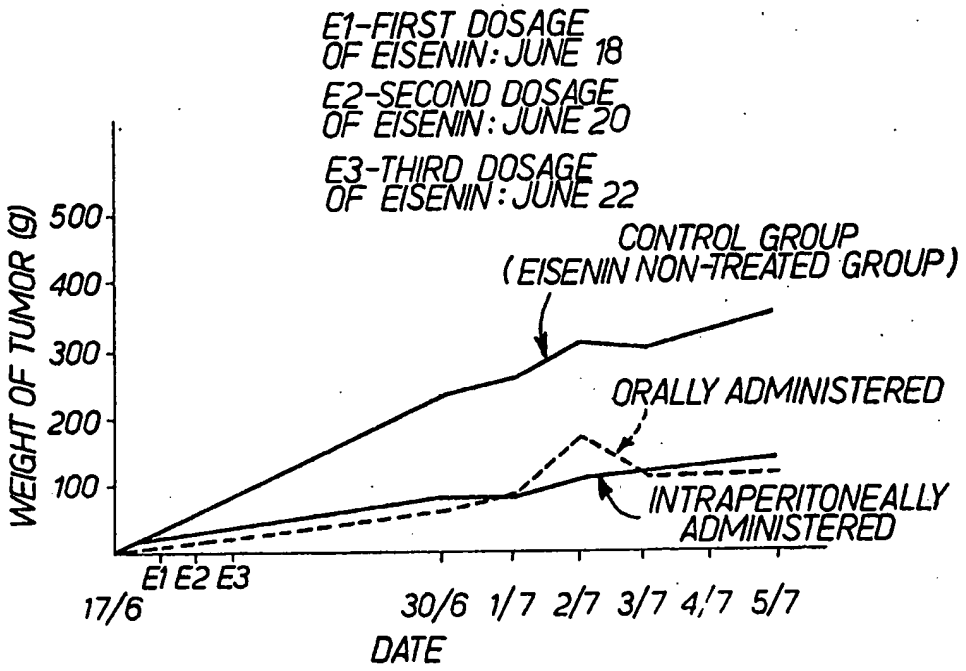
E1-FIRST DOSAGE OF EISENIN: JUNE 18
E2-SECOND DOSAGE OF EISENIN: JUNE 20
E3-THIRD DOSAGE OF EISENIN: JUNE 22
CONTROL GROUP (EISENIN NON-TREATED GROUP)
ORALLY ADMINISTERED
INTRAPERITONEALLY ADMINISTERED
WEIGHT OF TUMOR (g)
500
400
300
200
100
E1 E2 E3
17/6
30/6 1/7 2/7 3/7 4/7 5/7
DATE

FIG. 5.

SPECIFICATION

The tripeptide eisenin and carcinostatic compositions having an immunopotentiating carcinostatic effect which contain it

This invention relates to a carcinostatic agent having an immunopotentiating carcinostatic effect.

According to one aspect of this invention, there is provided the tripeptide Eisenia of the general formula:

L-Pyroglu-L-Glu-L-Ala

wherein Pyroglyu, Gly and Ala represent pyroglumatic acid, glumatic acid and alanine respectively, for use in a method for the treatment of cancer as a carcinostatic agent having an immunopotentiating carcinostatic effect.

The peptide of this invention is obtainable by extraction from the seaweed *Eisenia bicyclis*, and hence is generally formed herein Eisenin, or by synthesis, and acts as a carcinostatic agent having an immunopotentiating carcinostatic effect.

It has recently been found possible to synthesize Eisenin, although previously it was only extracted from the seaweed *Eisenia bicyclia*. Eisenin forms colourless long needles having a silk-thread gloss, shows a positive Biuret reaction, some shrinkage at a melting point of 180°C and decomposes on melting at 225–226°C, and yields an aqueous solution which is acidic.

It has now been found that Eisenin, whether of natural or synthetic origin, possesses an anti-tumour effect which is not a direct effect but which is manifested in such a way that the non-specific immunological ability of the vital body is increased. Eisenin is easy to synthesise because it has a simple tripeptide structure. Furthermore, since Eisenin is crystalline, it may be formed into fine powder, and since it is water soluble, it may be processed into various pharmaceutical forms, preferably in dosage unit form, such as injectable solutine, tablets, ointments and suppositories using pharmaceutically acceptable carriers and excipients commonly employed in pharmaceutical practice.

This invention therefore provides, in a second aspect, a pharmaceutical composition, which comprises the tripeptide Eisenin of the general formula

L-Pyroglu-l-Glu-L-Ala

wherein Pyroglu, Glu and Ala represent pyroglumatic acid, glumatic acid and alanine respectively, in association with a pharmacologically acceptable carrier.

For a better understanding of the invention and to show how the same may be carried into effect, reference will now be made by way of illustration only to the accompanying drawings, wherein.

Figure 1 is a graph showing the anti-tumour effect of the tripeptide Eisenin against the isologous allogenic tumor;

Figure 2 is a graph showing the life prolonging effect of the same;

Figure 3 is a graph showing the anti-tumor effect where the T-cells do not participate;

Figure 4 is a graph showing the life prolonging effect of the same; and

Figure 5 is a graph showing the anti-tumor effect of Eisenin against the isologous xenogenic tumor.

These figures will be referred to in the description of the results of tests carried out on animals using Eisenin which will now be reported. In the figures and in the tables which follow, the legends Eisenin Treated and Nontreated mean Eisenin administered and not administered respectively.

(1) Animal Experiments

The animals used as experimental subjects were Balb/C mice and nude mice, and the tumors used were induced by means of Colon (Balb/C Colon cancer) and Sarcoma 180.

a. 1,000,000 Colon 26 cells were transplanted subscutaneously into Balb/C mice. Eisenin was intraperitoneally administered to the mice at a rate of 5 mg per animal three times every other day. The weight of the tumor taken from the dead mice and the survival rate were determined. The results thereof are set forth in Fig. 1 and Fig. 2.

b. 1,000,000 Colon 26 cells were transplanted subcutaneously into nude mice, Eisenin was intraperitoneally administered to the mice at a rate of 5 mg per animal twice very other day, and the weight of the tumor on death and the survival rate were examined. The results are set forth in Fig. 3 and Fig. 4.

c. 1,000,000 Sarcoma 18 cells were transplanted subcutaneously into Balb/C mice, Eisenin was intraperitoneally and orally administered to the mice at a rate of 5 mg per animal three times every other day and the weight of the tumor on death was examined. The results are set

forth in Table 5.

d. 10^6 Sheep red blood cells (S R bc) were intravenously injected into Balb/C mice. Four days later, 10^8 sheep red cells and phosphate buffer solution (PBS) were subcutaneously injected into the sole of the left foot and at the same time the phosphate buffer solution (PBS) alone was subcutaneously injected into the sole of the right foot. A further two days later, the thicknesses of both feet were measured to examine the delayed hyper-sensitivity. The results are set forth in Table 1.

Table 1 Delayed Hyper-sensitivity

Group 1 T (+). E (+)				Group 2 T (+). E (-)				Group 3 T (-). E (-)				Group 4 T (-). E (+)			
L	R	L−R	$\frac{L-R}{R}$X100	L	R	L−R	$\frac{L-R}{R}$X100	L	R	L−R	$\frac{L-R}{R}$X100	L	R	L−R	$\frac{L-R}{R}$X100
1.59	1.57	0.02	1	1.91	1.69	0.22	13	1.90	1.85	0.05	3	2.09	1.86	0.23	12
1.56	1.54	0.02	1	1.83	1.83	0	0	1.95	1.85	0.10	5	2.18	1.98	0.20	10
1.87	1.68	0.19	11	1.91	1.80	0.11	6	2.08	1.92	0.16	8	1.84	1.86	0.02	0
1.95	1.49	0.46	31	1.86	1.67	0.19	11	1.89	1.78	0.11	6	1.93	1.81	0.12	7
1.75	1.67	0.08	5	1.78	1.69	0.09	5	1.91	1.82	0.09	5	1.87	1.72	0.15	9
1.54	1.57	0.03	0	1.96	1.72	0.24	14	2.01	1.78	0.23	13	1.93	1.61	0.32	20
1.58	1.54	0.04	3	1.71	1.52	0.19	13	1.97	1.91	0.06	3	1.87	1.88	0.01	0
1.90	1.65	0.25	15	1.82	1.69	0.13	8	1.99	1.80	0.19	11	1.93	1.87	0.06	3
-	-	-	-	1.83	1.87	0.04	0	1.92	1.82	0.10	5	-	-	-	-
-	-	-	-	1.77	1.77	0	0	1.92	1.75	0.17	10	-	-	-	-
Average			8	Average			7	Average			7	Average			8

Notes: 1. T(+) means tumor transplanted; T(-) means tumor not transplanted.
2. E(+) means Eisenin treated; E(-) means Eisenin non-treated.
3. L means the thickness of the left foot (mm);
R means the thickness of the right foot (mm).

(2) In Vitro Assay

a. Sensitivity Test

Colon 26 was tissue cultured, and brought into contact with Eisenin at various concentrations for 3 days. Tritium-thymidine, tritium-uridine and tritium-leucine were added to the culture medium and the inclusion of these compounds into the cells was examined to determine the inhibition index. Examination of the influence of Eisenin on the syntheses of DNA, RNA and protein traced through these labelled culture supplements yielded results which are set forth in Table 2.

Table 2 Sensitivity Test

Concentration of Eisenin (mg/ml)	1.0	0.1	0.01	0.001	0.0001	Control
Counts per Minute in the Case of Thymidine* (CPM)	140430	190120	207474	220010	231236	218622
	171170	203872	229569	213816	219336	220775
	163554	201151	240628	221170	224786	245962
	176167	223845	246273	213017	233612	226495
Average	170297	198381	238823	218332	229878	221964
I.I. (%)	23	11	8	2	0	
Counts per Minute in the Case of Uridine* (CPM)	27088	32774	37043	39415	32836	32553
	20903	25271	33211	31675	29585	31801
	19954	24222	31498	28671	24135	30304
	22463	26786	34408	33161	29958	27653
Average	21107	25426	33039	31169	30793	31553
I.I. (%)	33	19	0	0	2	
Counts per Minute in the Case of Leucine * (CPM)	6826	13311	12285	13620	12952	14674
	6150	12566	11020	14230	11936	11382
	—	8076	12097	13379	11808	12690
	6587	7076	11318	11355	10952	14350
Average	6521	11318	14478	13743	12232	13905
I.I. (%)	53	19	17	1	12	

Notes: (1) Inhibition Index (I.I.) is calculated from the following equation, and where I.I. exceeds 75%, it is judged effective.

$$I.I. = \frac{CPM\ (Control) - CPM\ (Drug)}{CPM\ (Control)} \times 100$$

(2) measurement was made on a scintillation counter

* tritium labelled

b. Cytotoxicity Test

15 Mg of Eisenin were intraperitoneally administered to Balb/C mice and starch was administered to a control group. Both test and control mice had implanted, as target cells, BW 5174 (thymoma of AKR mouse) cells. The spleen cells and the intraperitonel cells of sacrificed mice were respectively divided into the adherent cells and the non-adherent cells, and cytoxicity was examined in each case. The results are set forth in Table 3.

Table 3 Cytotoxicity Test

	Eisenin Administered	Control Group
Spleen Cells		
Total	24%	23%
Non-adherent Cells (Natural Killer)	13%	15%
Adherent Cells (Macrophage)	10%	10%
Intraperitoneal Cells		
Total	24%	-8%
Non-adherent Cells (Natural Killer)	32%	2%
Adherent Cells (Macrophage)	17%	-5%

Notes: (1) Percent Cytotoxicity (%) in Table 3 is based on the following equation:

(2) Measurement is made by ^{51}Cr release assay.

(3) Acute Toxicity Test

9 Balb/C mice (average body weight 20 g) were subjected to intraperitoneal administeration of Eisenin. The results obtained are set forth in Table 4.

Table 4 Acute Toxicity Test

		No. of Deaths
Dosage of Eisenin (g/kg)	6	7 / 9
	3	1 / 9

The results of the assays described above may be summarised thus. Table 1 and Table 2

show that in the case of such an isologous allogenic tumor as Colon 26 administered to Balb/C mice, a significant difference between the Eisenin treated group and the Eisenin non-treated group is observed thus indicating an anti-tumor effect for Eisenin; further, as shown in Figs. 3 and 4, similar results have been obtained in the case of the nude mice free from T cells; moreover a similar anti-tumor effect is recognized in the case of such isologuous xenogenic tumor as Sarcoma 180 against Balb/C mice as shown in Fig. 5. On the contrary, as can be seen from the results of the delayed hyper-sensitivity test on Balb/C mice shown in Table 1, there is hardly any difference between the average values for the four groups of combinations corresponding to the presence and absence of tumor transplantation and the presence and absence of treatment with Eisenin. Hence Eisenin has no effect on activation of immunological actions in which T cells participate. The sensitivity test results shown in Table 2, show that the inhibition index (I.I.) is low which indicates no inhibition effect by Eisenin; this means that Eisenin has no direct effect on tumor cells. In addition, in the results of the cytotoxicity test shown in Table 3, although there is no significant difference in the case of the spleen cells, there is a significant difference in the case of intraperitoneal cells, and as a result, it can be recognized that the immunity imparting effect of Eisenin is mainly due to the activation of the adherent cells and the non-adherent cells in the peritoneal cavity. Furthermore, the acute toxicity test shows the LD_{50} of Eisenin to be in the vicinity of 5 g/kg. The effective dosage as estimated from the various test results above is suitably about 15 mg per 20 g body weight, taking the average body weight of Balb/C mice used in the experiments to be 20 g, that is, about 75 mg/kg. It is believed to be appropriate to administer Eisenin to a subject in this amount in several doses.

CLAIMS

1. The tripeptide Eisenia of the general formula:

L-Pyroglu-L-Glu-L-Ala

wherein Pyrogly, Glu and Ala represent pyroglumatic acid, glumatic acid and alanine respectively, for use in a method for the treatment of cancer as a carcinostatic agent having an immunopotentiating carcinostatic effect.

2. The carcinostatic agent of Claim 1 wherein the Eisenin is extracted from *Eisenia bicyclis.*

3. The carcionstatic agent of Claim 1 wherein the Eisenin is synthetically obtained.

4. A pharmaceutical composition, which comprises tripeptide Eisenin of the general formula

L-Pyroglu-L-Glu-L-Ala

wherein Pyroglu, Glu and Ala represent pyroglytamic acid, glutamic acid and alanine respectively, in association with a pharmacologically acceptable carrier.

5. A pharmaceutical composition as claimed in Claim 4, which is made up into a sterile injectable solution.

6. A pharmaceutical composition as claimed in Claim 4, substantially as described herein.

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